Anal. Calcd for $C_5H_{10}N_2O_2$: C, 46.14; H, 7.74; N, 21.53. Found: C, 45.96; H, 7.90; N, 21.28.

Method B. To 0.078 g (0.7 mmol) of **4** placed in a dry, three-necked flask under nitrogen and cooled to 0 °C was added 2 mL (2 mmol) of a 4 °C solution of 1 M BH_3-THF through a septum cap. The mixture was stirred for 15 min at 0 °C and allowed to warm up to room temperature. A colorless gel was formed. After this mixture was allowed to sit for 2.5 h, 1 mL of water was added followed by the dropwise addition of 3 mL of aqueous 1 N NaOH and 3 mL of 30% H_2O_2. The reaction mixture was stirred at room temperature for 1 h, reduced to dryness, and coevaporated several times with methanol. The residue was extracted with methanol and purified on a preparative silica gel TLC plate with CH_2Cl_2–MeOH (4:1) to afford 0.073 g (80%) of **3** (R_f 0.39) as a crystalline material identical in all respects with the compound obtained through method A.

Perhydro-1,3-diazepin-2-one (1). A solution of **4** (0.050 g, 0.446 mmol) in 10 mL of methanol was hydrogenated at 35 psi for 9 h in the presence of 20 mg of 10% Pd/C. The catalyst was removed and the filtrate reduced to dryness to provide colorless crystals of **1** identical with an authentic sample prepared according to ref 21; mp 166–168 °C [lit.[21] mp 166–170 °C].

Acknowledgment. The authors thank Dr. Herman Yeh, NIAMDD, NIH, for his help in providing the FT NMR data. We also thank Mr. Richard Fuller of this department, who synthesized large-scale batches of dimethyl β-ketoadipate, and Dr. R. Richter from the Upjohn Co. for his helpful comments.

Registry No. 1, 19055-93-7; **2**, 72331-38-5; **3**, 72331-39-6; **4**, 72331-40-9; **6a**, 5457-44-3; **7a**, 26954-91-6; **7b**, 72331-41-0; **7c**, 72331-42-1; **7d**, 72331-43-2; **7e**, 72331-44-3; **7f**, 72331-45-4; **8a**, 72331-46-5; **8b**, 72331-47-6; **9a**, 72331-48-7; **9b**, 72331-49-8; **11**, 40794-71-6; **12**, 40794-72-7; carbonyl sulfide, 463-58-1.

A Thiol-Containing Ester Side Chain in a Sesquiterpene Lactone from *Eupatorium mikanioides*. Absolute Configuration of Deacetyleupaserrin and Its Congeners[1]

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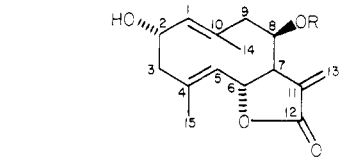
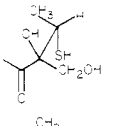
Extraction of *Eupatorium mikanioides* Chapm. yielded a group of 2-hydroxy-8-(acyloxy)-*trans,trans*-1-(10),4-germacradienolides related to the antileukemic sesquiterpene lactone deacetyleupaserrin (**1c**) which was also isolated. Absolute configurations were established by X-ray crystallography of one of the constituents (**1e**) which had an unprecedented thiol-containing ester side chain. The flavone eupatorin was also isolated.

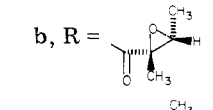
As part of our continuing study[2-4] of *Eupatorium* species *sensu stricto* which elaborates a number of sesquiterpene lactones with cytotoxic and antitumor activity, we have investigated *Eupatorium mikanioides* Chapm., a diploid which is restricted to the coastal areas of peninsular Florida.[5] This has resulted in the isolation of a family of six germacradienolides **1a–f**. **1c**, which was the most abundant lactone constituent (ca. 0.15% of dry weight), is the antileukemic lactone deacetyleupaserrin which has been isolated previously from *E. semiserratum*,[6] *Helianthus pumilus*,[7] and *Helianthus mollis*.[8] X-ray analysis of one of the minor constituents, undertaken to establish the relative stereochemistry of the various ester side chains, led to the discovery of an unusual sulfur-containing ester residue in **1e** and was used to deduce the previously hypothetical absolute stereochemistry of deacetyleupaserrin as well as that of its congeners.



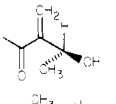
1a, R = H



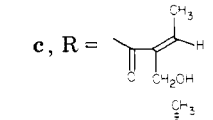
e, R =



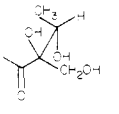
b, R =



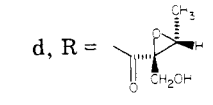
f, R =



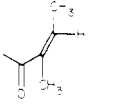
c, R =



g, R =



d, R =



h, R =

We deal first with the sesquiterpene portion common to the various lactones whose nature was deduced by NMR spectrometry at 270 MHz. ^1H chemical shifts and coupling constants are given in Table I and parallel those of desacetyleupaserrin (**1c**)[7,9] for the hydrogen atoms of the ses-

(1) Work at the Florida State University was supported in part by a grant from the U.S. Public Health Service through the National Cancer Institute (CA-13121).

(2) Herz, W.; de Groote, R; Murari, R.; Kumar, N.; Blount, J. F. *J. Org. Chem.* **1979**, *44*, 2784. This article contains references to earlier work.

(3) Herz, W.; Govindan, S. V.; Blount, J. F. *J. Org. Chem.* **1979**, *44*, 2999.

(4) Herz, W.; Murari, R.; Govindan, S. V. *Phytochemistry* **1979**, *18*, 1337.

(5) Sullivan, V. I. Ph.D. Dissertation, Florida State University, Aug 1972.

(6) Kupchan; S. M.; Fujita, T.; Maruyama, M.; Britton, R. W. *J. Org. Chem.* **1973**, *38*, 1260.

(7) Herz, W.; de Groote, R. *Phytochemistry* **1977**, *16*, 1307. In formulas **1a–c** of this reference the configuration of deacetyleupaserrin and its derivatives at C-6 and C-8 is reproduced incorrectly.

(8) Ohno, N.; Mabry, T. J. *Phytochemistry* **1979**, *18*, 1003.

(9) In Table I of ref 7, assignments of H-14 and H-15 were inadvertently interchanged.

0022-3263/80/1945-0489$01.00/0

Table I. ^{1}H NMR Spectra of *E. mikanioides* Constituents[a]

	1a	1b	1d	1e	1f
H-1	4.95 d, br (10, 1.5)	5.00 d, br	5.04 d, br	5.03 d, br	5.03 d, br
H-2	4.77 dt (6, 10)	4.77 dt	4.74 dt	4.75 dt	4.74 dt
H-3a	2.71 dd (6, 10.5)	2.76 dd	2.73 dd	2.76 dd	2.73 dd
H-3b	2.08 t (10.5)	2.08 t	2.12 t	2.12 t	2.11 t
H-5	4.92 d, br (10.5, 2)	4.98 d, br	4.96 d, br	4.95 d, br	4.99 d, br
H-6	5.19 dd (10.5, 8)	5.03 dd	5.08 dd	5.12 dd	5.10 dd
H-7	2.81 m (8, 1.5, 3.2, 3)	2.90 m	3.01 m	3.00 m	2.98 m
H-8	4.62 dd, br (1.5, 5.5, 2.5)	5.87 dd, br	5.91 dd, br	5.92 dd, br	5.82 dd, br
H-9a	2.71 dd (14, 5.5)	2.81 dd	2.79 dd	2.82 dd	2.83 dd
H-9b	2.29 dd (14, 2.5)	2.30 dd	2.42 dd	2.42 dd	2.37 dd
H-13a	6.38 d (3.2)	6.32 d	6.31 d	6.36 d	6.30 d
H-13b	5.57 d (3)	5.57 d	5.60 d	5.67 d	5.60 d
H-14[b]	1.69 br (2)	1.61 br	1.61 br	1.65 br	1.52 br
H-15[b]	1.77 d (2)	1.82 br	1.82 br	1.81 br	1.80 br
H-3′		3.00 q (5)	3.26 q (5.5)	4.10 q (7)	4.61 q (7)
H-4′[b]		1.23 d (5)	1.22 d (5.5)	1.45 d (7)	1.35 d (7)
H-5′[b]		1.52[b]	3.82 d (13)	3.68 d (12)	6.05 br (1)
			4.12 d (13)	3.77 d (12)	5.89 br (1)

[a] Run at 270 MHz in $CDCl_3$ with Me_4Si as internal standard. Values are in parts per million. Unmarked signals are singlets. Figures in parentheses are coupling constants in hertz and are not repeated in the same row. Values for 1c are given in ref 8 (see also ref 10). [b] Three-proton intensity.

Table II. ^{13}C NMR Spectra of *E. mikanioides* Constituents[a]

	1a	1b[b]	1c	1c[b]	1d	1e	1f
C-1	132.91 d	134.54 d	134.97 d	134.05 d	134.74 d	134.51 d	133.27 d
C-2	67.78 d	69.26 d	67.82 d	69.14 d	67.79 d	68.09 d	67.90 d
C-3	48.70 t	48.66 t	48.73 t	48.77 t	48.64 t	48.70 t	48.88 t
C-4	141.49	143.01	142.28	143.07	142.23	142.18	142.34
C-5	129.32 d	129.28 d	128.72 d	129.18 d	128.78 d	128.25 d	128.82 d
C-6	74.74 d	75.24 d	75.50 d	75.98 d	75.04 d	75.01 d	75.41 d
C-7	53.11 d	52.83 d	51.91 d	53.04 d	51.56 d	51.70 d	51.96 d
C-8	70.42 d	72.80 d	71.52 d	71.76 d	72.91 d	73.23 d	72.14 d
C-9	47.13 t	44.23 t	43.18 t	43.98 t	43.47 t	42.65 t	43.16 t
C-10	134.79	134.24	132.68	134.82	132.81	133.42	132.45
C-11	138.76	136.26	137.19	136.51	136.59	136.22	137.32
C-12	169.76	169.27	169.00	169.82	168.84	169.20	169.05
C-13	119.89 t	121.36 t	120.34 t	121.53 t	120.85 t	121.33 t	120.24 t
C-14	20.26 q	20.34 q	19.44 q	19.87 q	19.53 q	19.54 q	19.71 q
C-15	18.17 q	18.78 q[c]	18.25 q	18.82 q	18.30 q	18.58 q[c]	18.38 q
C-1′		168.53	165.22	165.89	167.16	171.30	164.97
C-2′		59.42	132.32	131.49	64.11	81.26	145.33
C-3′		60.13 d	137.58	140.75	54.87 d	59.53 d	64.52 d
C-4′		13.69 q	15.09 q	15.90 q	13.48 q	19.94 q[c]	23.25 q
C-5′		19.16 q[c]	61.91 t	64.00 t	61.88 t	65.24 t	123.33 t

[a] Run at 67.09 MHz in Me_2SO-d_6 unless specified otherwise with Me_4Si as internal standard. Values are in parts per million. Unmarked signals are singlets. [b] In $CDCl_3$. [c] Assignments may be interchanged.

quiterpene moiety, except for H-8 of **1a** which exhibited the expected upfield shift; the decoupling procedure and the arguments used to deduce the gross structure and relative stereochemistry in each case were the same as those employed previously[2,7] and will not be detailed. ^{13}C NMR spectra are listed in Table II; all ring multiplets were identified by single-frequency off-resonance decoupling except for the doublets of C-1 and C-5 which because of superposition of the H-1 and H-5 signals could not be decoupled selectively. We tentatively ascribe the doublet at lower field to C-1 by assuming that it is shifted downfield from its usual position near 127–129 ppm by the β effect of the C-2 hydroxyl group.[10] As usual the C-10 singlet is at higher frequency than that of C-4 and is additionally shielded (cf. C-10 of costunolide at 137 ppm) by the axial ester substituent.

That the sesquiterpene portions of all compounds were identical was confirmed chemically by hydrolysis of all ester lactones to **1a**. CD spectrometry suggested that the absolute configuration of this portion was 2*S*, 6*R*, 7*R*, 8*R* because all compounds exhibited negative Cotton effects near 250 nm (n,π^* transition of α,β-unsaturated lactone)[11] and strongly positive Cotton effects in the short wavelength region (combination of π,π^* transition of lactone and transannular interaction of ring double bonds).[14] Conclusive evidence regarding the absolute configurations is provided below.

The gross structures of the ester side chains in **1b**, **1d**, and **1f** were established by a combination of mass, ^{1}H, and

(10) This assignment and some others differ somewhat from several in a report on the constituents of *Helianthus mollis*.[8]

(11) Although we earlier[7] reported the CD curve of deacetyleupaserrin, we did not then comment on the absolute configuration because it was not known whether the Stöcklin–Waddell–Geissman rule[12] was applicable to this compound in view of the presence of an α,β-unsaturated ester side chain. Comparison of **1a** with **1b–f** shows that in this series the presence of an inherently symmetric but asymmetrically perturbed α,β-unsaturated ester chromophone on C-8 has not affected the sign of the diagnostically valuable α,β-unsaturated lactone band which has been associated with the chirality of the C=C—C=O chromophone. Hence in this series the CD curve seems to provide a criterion of absolute stereochemistry (vide infra).

(12) Stöcklin, W.; Waddell, T. G.; Geissman, T. A. *Tetrahedron* **1970**, *26*, 2397.

(13) Beecham, A. F. *Tetrahedron* **1972**, *28*, 5543.

(14) Suchy, M.; Dolejš, L.; Herout, V.; Šorm, F.; Snatzke, G.; Himmelreich, J. *Collect. Czech. Chem. Commun.* **1969**, *34*, 229.

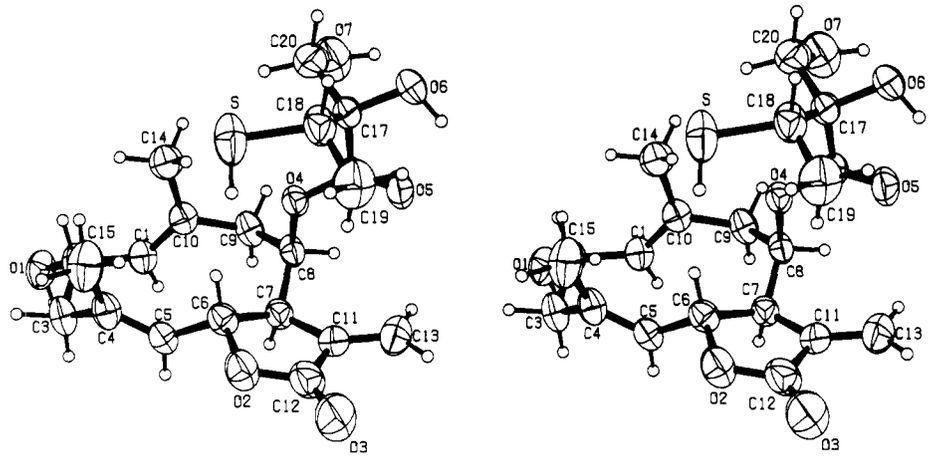


Figure 1. Stereoscopic view of **1e**.

^{13}C NMR spectrometry (see Tables I and II and the Experimental Section). For the remaining sesquiterpene lactone (later shown to be **1e**), neither electron-impact nor chemical-ionization mass spectrometry furnished satisfactory evidence concerning the molecular ion, but ^{1}H and ^{13}C NMR spectra (Tables I and II) seemed consonant with the previously unencountered ester residue of the sesquiterpene lactone **1g**.[15] The mass spectrum, however, contained a reasonably strong peak corresponding to an acylium ion, $C_5H_7O_2$, formed, it was assumed, by loss of water from the ester portion of **1g**. Additional plausibility was conferred on structure **1g** by the presence of the congeners **1d** and **1f**.

As regards the stereochemistry of the ester portions, that of **1b** was definitely an epoxyangelate (2′*R*,3′*R* or 2′*S*,3′*S*) because of the coincidence of the chemical shifts of H-3′, H-4′, and H-5′ and the shifts of the side chain ^{13}C signals with those of authentic epoxyangelates (cf. melampodin)[16] as well as the demonstration of an NOE (~7%) involving H-3′ and H-5′. By analogy, the ester side chain of **1d** was assumed to be derived by epoxidation of the sarracenoyl residue of **1c**. If this were so, enzyme-induced nucleophilic attack at C-3′ with inversion would have produced the side-chain stereochemistries shown in the formulas for **1f** and **1g**.[17]

To verify this supposition and to deduce the absolute stereochemistry of the ester side chains (on the assumption that the absolute configuration of the sesquiterpene moiety could be deduced from the CD curves), we undertook an X-ray analysis of the putative **1g**. This led to the surprising discovery that the C-3′ atom of this sesquiterpene lactone carried a sulfhydryl instead of a hydroxyl group. The unexpected result provided a bonus, however, in that it permitted the independent verification, by the anomalous dispersion method, of the absolute configuration of the sixth lactone as **1e** and, by inference, the deduction that the absolute configurations of the other lactones were also as depicted in the formulas.

Crystal data for **1e** are listed in the Experimental Section.[7] Tables IV and V, containing final atomic and anisotropic thermal parameters, and Tables VI–VIII, listing bond lengths, bond angles, and selected torsion angles, are available as supplementary material (see Supplementary Material available paragraph). Figure 1 is a stereoscopic drawing of the molecule which shows the absolute configuration (2*S*,6*R*,7*R*,8*R*,2′*S*,3′*S*). Since all *E. mikanioides* constituents have been correlated via **1a**, the absolute configuration at C-2, C-6, C-7, and C-8 of **1a–d** and **1f** is established as well, and, on the basis of the arguments presented earlier, the absolute configurations of the ester side-chain carbons are 2′*R*,3′*R* for **1b**, 2′*R*,3′*R* for **1d**, and 3*S* for **1f**.[18]

Table III. Lactone Ring Torsion Angles (deg) of 1e

C(6)-O(2)-C(12)-C(11)	ω_1	−7.2
C(13)-C(11)-C(12)-O(3)	ω_2	−9.4
C(11)-C(7)-C(6)-O(2)	ω_3	−7.9
C(5)-C(6)-C(7)-C(8)	ω_4	92.1

The conformation of the germacradiene moiety of **1e** is not significantly different from that deduced by X-ray analysis for other *trans,trans*-1(10), 4-germacradien-6,12-olides.[2,19-25] The magnitudes of the lactone torsion angles (Table III) are similar to those of eupatolide,[21] costunolide,[20] and tamaulipin A;[22] the sign of ω_2 (negative) is paired with that of ω_3, and thus, at least in this series, the chirality of the lactone chromophore correlates with the sign of the Cotton effect in the 250-nm region.[13] The conformation of the ester residue appears to be such so as to permit

(15) A recent communication reports the presence of the acetylated ester side chain of **1g** in ursiniolide B, but without specification of stereochemistry: Samek, Z.; Holub, M.; Rychlewska, V.; Grabarczyk, H.; Drozdz, B. *Tetrahedron Lett.* **1979**, 2691.

(16) (a) Fischer, N. H.; Wiley, R.; Wander, J. D. *J. Chem. Soc., Chem. Commun.* **1972**, 137. (b) Neidle, S.; Rogers, D. *Ibid.* **1972**, 140. (c) Bhacca, N. S.; Wehrli, F. W.; Fischer, N. H. *J. Org. Chem.* **1973**, *38*, 3619.

(17) Cf. the formation of enhydrin bromohydrin: Kartha, G.; Go, K. T.; Joshi, B. S. *J. Chem. Soc., Chem. Commun.* **1972**, 1327.

(18) The structure deduced for **1b** which is a gum is the same as that suggested recently[8] for mollisorin B, a crystalline constituent of *Helianthus mollis*. A difference in physical state would a priori not be particularly surprising because lactones of type 1, although relatively high melting, frequently crystallize with difficulty. Thus deacetyleupaserrin **1c** is crystalline although it was initially[6] reported as a gum. However, a diol, presumably **1a**, obtained in poor yield by hydrolysis of mollisorin A (**1h**) and mollisorin B reportedly[8] melts at 157–159 °C while our diol (**1a**) from *E. mikanioides* melts at 184–186 °C (see Experimental Section). The spectral properties of **1a** and those reported for the mollisorin hydrolysis product agree reasonably well, but a direct comparison under identical conditions of the 270-MHz NMR spectra of **1b** and a small sample of mollisorin B kindly supplied by Professor Mabry showed minor though definite chemical shift differences not dependent on concentration (see Experimental Section), and seeding our **1b** with mollisorin B did not result in crystallization. Consequently mollisorin B differs from **1b**, possibly in the absolute configuration of its ester side chain.

(19) Bovill, M. J.; Cox, R. J.; Cradwick, P. D.; Guy, M. H. P.; Sim, G. A.; White, D. N. J. *Acta Crystallogr., Sect. B* **1976**, *32*, 3203.

(20) Cox, P. J.; Sim, G. A.; *J. Chem. Soc., Perkin Trans. 2* **1977**, 255.

(21) McPhail, A. T.; Onan, K. D., *J. Chem. Soc., Perkin Trans. 2* **1975**, 1798.

(22) Witt, M. E.; Watkins, S. F. *J. Chem. Soc., Perkin Trans. 2*, **1978**, 204.

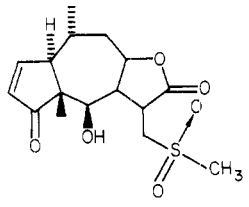
(23) Hull, S. E.; Kennard, O.; *Cryst. Struct. Commun.* **1978**, *7*, 85.

(24) Jones, P. G.; Kennard, O.; *Acta Crystallogr., Sect B* **1977**, *35*, 1273.

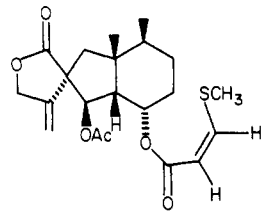
(25) Gopalakrishna, E. M.; Watson, W. H.; Hoeneisen, M.; Silva, M. *J. Cryst. Mol. Struct.* **1977**, *7*, 49.

hydrogen bonding between the two hydroxyl functions and places the sulfhydryl group over the ten-membered ring. Whether this conformation or any other in solution is responsible for the unusual chemical shift of C-3′,[26] which was at least in part responsible for our failure to suspect the presence of sulfur in **1e**, is questionable.

Only two other sulfur-containing sesquiterpene lactones are recorded in the literature, sulferalin (**2**) from the roots



2



3

of a Japanese cultivar of *Helenium autumnale*[27] and *S*-fukinolide (**3**) from *Petasites japonicus*.[28] Sulfur-containing esters are very rare among terpenoids, and the ester side chain of **1e** is unprecedented.

Experimental Section

Isolation of Compounds. Above-ground parts of *Eupatorium mikanioides* Chapm., collected by Mr. Douglas Gage in the St. Marks Wildlife Refuge, Wakulla Co., Florida on August 15, 1978 (DG No. 11, on deposit in Florida State University herbarium; 8.5 kg), was extracted with $CHCl_3$ and worked up in the usual manner.[29] The crude gum, 300 g, was adsorbed on 350 g of silicic acid (Mallinckrodt, 100 mesh) and chromatographed over 3.5 kg of the same adsorbent packed in toluene–$CHCl_3$ (1:1), 1-L portions of eluent being eluted in the following order: 1–6, Tol–$CHCl_3$ (1:1); 7–12, $CHCl_3$; 13–20, $CHCl_3$–MeOH (99:1); 21–28 $CHCl_3$–MeOH (49:1); 29–34, $CHCl_3$–MeOH (9:1), 35–38, $CHCl_3$–MeOH (4:1).

Fractions 1–6 were waxy material from which no pure substances were isolated. Fractions 7–12 which showed a major spot on TLC were combined and rechromatographed over silica gel. The $CHCl_3$ portions of the eluate yielded **1b** as a gum, 700 mg, which could not be induced to crystallize: $[\alpha]_D$ +67.7° (*c* 0.204, $CHCl_3$); CD (MeOH) $[\theta]_{255}$ −7170, $[\theta]_{210}$ +62 700 (last reading); IR ($CHCl_3$) 3480, 1760, 1735, 1650, 1260, 955, 915 cm^{-1}.

Anal. Calcd for $C_{20}H_{26}O_6$: mol wt 362.1728. Found mol wt (mass spectrum) 362.1743 (0.3% relative intensity).

Other significant peaks in the high-resolution mass spectrum were at *m/e* (relative intensity) 263 ($C_{15}H_{19}O_4$, 5.6), 262 ($C_{15}H_{18}O_4$, 16.5), 249 ($C_{14}H_{17}O_4$, 8.3), 247 ($C_{15}H_{19}O_3$, 9.1), 246 ($C_{15}H_{18}O_3$, 37.7), 245 ($C_{15}H_{17}O_3$, 11.3), 244 ($C_{15}H_{16}O_3$, 23.8), 231 ($C_{14}H_{15}O_3$, 24.9), 228 ($C_{15}H_{16}O_2$, 21.8), 213 ($C_{14}H_3O_2$, 17.5), 204 ($C_{13}H_{16}O_2$, 26), 203 ($C_{13}H_{15}O_2$, 38.1), 202 ($C_{13}H_{14}O_2$, 36.1), 201 ($C_{13}H_{13}O_2$, 20.9), 189 ($C_{12}H_{13}O_2$, 27.8), 164 ($C_{10}H_{12}O_2$, 41.5), 163 ($C_{10}H_{11}O_2$, 89.9), 162 ($C_{10}H_{10}O_2$, 53.5), 161 ($C_{10}H_9O_2$, 58.9), 157 ($C_{12}H_{13}$, 43.2), 135 ($C_9H_{11}O$, 95.3), 134 ($C_9H_{16}O$, 69.1), 117 (C_9H_9, 76.8), 107 (C_8H_{11}, 92.9), 106 (C_8H_{10}, 69.6), 105 (C_8H_9, 100), 99 ($C_5H_7O_2$, 44.1).

Fractions 13–20 contained two major constituents which were separated by preparative TLC (EtOAc–hexane, 1:1). The upper band yielded eupatorin, 200 mg, mp 195–197 °C, identical with an authentic specimen; the lower band gave 3 g of **1f** which could not be induced to crystallize: $[\alpha]_D$ +80.5° (*c* 0.23, $CHCl_3$), CD (MeOH) $[\theta]_{256}$ −7170, $[\theta]_{209}$ 89 600 (last reading); IR ($CHCl_3$) 3420, 1760, 1725, 1660, 1640, 1630, 1260, 920 cm^{-1}.

Anal. Calcd for $C_{20}H_{26}O_6$: mol wt 362.1728. Found: mol wt (mass spectrum) 362.1698.

Additional significant peaks in the high-resolution mass spectrum were preparative *m/e* 347 ($C_{19}H_{23}O_6$, 1.4), 344 ($C_{20}H_{24}O_5$, 0.3), 300 ($C_{18}H_{20}O_4$, 2.6), 264 ($C_{15}H_{20}O_4$, 1.5), the remainder being similar to that of **1b**. The base peak was at *m/e* 99 ($C_5H_7O_2$).

Fractions 21–25 contained two compounds which were separated by preparative TLC (EtOAc–hexane, 7:3, two developments). The upper band yielded **1a**, 1 g, which crystallized from $CHCl_3$–MeOH as colorless cubes: mp 184–186 °C; insoluble in $CHCl_3$, partially soluble in MeOH; $[\alpha]_D$ +196.5° (*c* 0.098, py); CD (MeOH) $[\theta]_{256}$ −7840, $[\theta]_{214}$ +78 400 (last reading); IR (KBr) 3430, 1750, 1660, 1650, 1635, 835 cm^{-1}.

Anal. Calcd for $C_{15}H_{20}O_4$: mol wt 264.1361. Found: mol wt (mass spectrum) 264.1367.

The next significant peak in the high-resolution mass spectrum MS was at *m/e* (relative intensity) 246 ($C_{15}H_{18}O_3$, 15); the rest of the spectrum was similar to that of **1b** but did not exhibit peaks resulting from an ester side chain.

The lower band yielded 2 g of deacetyleupaserrin (**1c**) which crystallized from EtOAc–hexane on prolonged standing as colorless cubes, mp 132–135 °C, and was identical with an authentic sample.[7] Fractions 26–28 were essentially homogeneous and yielded an additional 10 g of **1c**, the most abundant lactone constituent of *E. mikanioides*.

Fractions 29–34 contained two constituents which were separated by preparative TLC (EtOAc–hexane) by double development. The upper band yielded **1d**: colorless rods from $CHCl_3$–MeOH (2:3); yield 0.5 g; mp 170–172 °C; $[\alpha]_D$ +97.8° (*c* 0.027, py); CD (MeOH) $[\theta]_{256}$ −6550, $[\theta]_{212}$ +67 400 (last reading); IR (KBr) 3400, 1760, 1740, 1650, 1250, 905, 820 cm^{-1}.

Anal. Calcd for $C_{20}H_{26}O_7$: C, 63.48; H, 693; mol wt 378.1678. Found: C, 63.28; H, 6.94; mol wt (mass spectrum) 378.1681.

Other peaks in the high-resolution mass spectrum were similar to those in the spectrum of **1b** except for the presence of a side-chain peak at *m/e* (relative intensity) 97 ($C_5H_5O_2$, 16.9) and the absence of a peak at *m/e* 99.

The lower band yielded 800 mg of **1e** which was crystallized from $CHCl_3$–MeOH, mp 180–181 °C, with a slightly pungent odor: insoluble in $CHCl_3$, partially soluble in MeOH; $[\alpha]_D$ +102° (*c* 0.02, py); CD (MeOH $[\theta]_{255}$ −6860, $[\theta]_{214}$ +68 000 (last reading); IR 3440, 3220, 1720, 1665, 1655, 1260, 960, 830 cm^{-1}. The high-resolution mass spectrum did not exhibit a peak for the molecular ion but exhibited peaks at *m/e* (relative intensity) 265 ($C_{15}H_{21}O_4$, 3.5), 264 ($C_{15}H_{20}O_4$, 3.6), 249 ($C_{14}H_{17}O_4$, 1.1), 247 ($C_{15}H_{19}O_3$, 27.1), 246 ($C_{15}H_{18}O_3$, 25.4), 233 ($C_{14}H_{17}O_3$, 9.8), 231 ($C_{14}H_{15}O_3$, 9.2), 230 ($C_{15}H_{18}O_2$, 8.4), 229 ($C_{15}H_{17}O_2$, 20.5), 228 ($C_{15}H_{16}O_2$, 19), 218 ($C_{14}H_{18}O_2$, 23.3), 204 ($C_{13}H_{16}O_2$, 21.2), 203 ($C_{13}H_{15}O_2$, 41.6), 202 ($C_{13}H_{14}O_2$, 41.3), 175 ($C_{12}H_{15}O_1$, 21.8), 164 ($C_{10}H_{12}O_2$, 23.7), 163 ($C_{10}H_{11}O_2$, 100), 161 ($C_{10}H_9O_2$, 34.4), 135 ($C_9H_{11}O$, 69.3), 105 (C_8H_9, 53.2), 99 ($C_5H_7O_2$, 12.8).

Anal. Calcd for $C_{20}H_{28}O_7S$: C, 58.23; H, 6.84. Found: C, 57.98; H, 6.66.

The NMR spectra of **1b** and mollisorin B were determined consecutively under identical conditions in $CDCl_3$ solution and were not superimposable, although very similar. Chemical shifts for mollisorin B were 6.33 (d, H-13a), 5.81 (dd, br, H-8), 5.60 (d, H-13b), 5.09 (dd, H-6), 5.02 (d, br H-5), 4.75 (dt, H-1), 3.03 (q, H-3′), 2.97 (m, H-7), 2.79 (dd, H-9a), 2.73 (dd, H-3a), 2.34 (dd, H-9b), 2.11 (H-3b), 1.80 (H-15), 1.57 (H-14), 1.50 (H-5′), and 1.24 ppm (d, H-4′). Coupling constants were the same as for **1b** except for $J_{7,8}$ which was approximately 2.5 Hz.

Hydrolysis of 1b–f. A 25-mg sample of **1f** was stirred with 20 mg of KOH in 2 mL of H_2O at ambient temperature for 15 h. The solution was acidified with dilute HCl, diluted with water, and extracted with EtOAc. Evaporation of the dried organic layer yielded a gum which was purified by preparative TLC ($CHCl_3$–MeOH, 9:1) and was crystallized from $CHCl_3$–MeOH to give 12 mg of **1a**, mp 183–184 °C, identical with material isolated from the plant in all respects (NMR, TLC). Hydrolysis of 25 mg of **1b**, **1c**, **1d**, and **1e** was carried out similarly with identical results; in each case, **1a** was obtained in crystalline form.

X-ray Analysis of 1. Single crystals of **1e** were prepared by slow crystallization from $CHCl_3$–MeOH (2:3). They belonged to space group $P2_1$, with a = 11.927 (5) Å, b = 7.525 (4) Å, c = 11.461 (4) Å, β = 95.20 (3)°, and d_{calcd} = 1.337 g cm^{-3} for Z = 2 ($C_{20}H_{28}O_7S$;

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mol wt 412.50). The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu Kα radiation, θ–2θ scans, pulse-height discrimination). A crystal measuring approximately 0.15 × 0.20 × 0.8 mm was used for data collection; the data were corrected for absorption (μ = 17.1 cm^{-1}). A total of 1502 reflections were measured for $\theta < 57°$, of which 1452 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[30] and was refined by full-matrix least-squares methods. In the final refinement, anisotropic thermal parameters were used for the heavier atoms, and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure-factor calculations, but their parameters were not refined. The presence of sulfur permitted determination of the absolute configuration which is shown in Figure 1. Both enantiomers were refined. The final weighted R values were 0.0452 for the configuration illustrated and 0.0490 for its antipode, thus conclusively establishing the absolute configuration of **1e**. The final difference map had no peaks greater than ±0.3 Å^{-3}.

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Registry No. **1a**, 72229-33-5; **1b**, 72229-34-6; **1c**, 38456-39-2; **1d**, 72229-35-7; **1e**, 72229-36-8; **1f**, 72229-37-9; **1g**, 72229-38-0; **1h**, 72229-39-1; mollisorin B, 72258-24-3.

Supplementary Material Available: Final atomic parameters (Table IV), anisotropic thermal parameters (Table V), bond lengths (Table VI), bond angles (Table VII), and selected torsion angles (Table VIII) of **1e** (6 pages). Ordering information is given on any current masthead page.

Sesquiterpene Lactones of *Hymenoxys insignis*. X-ray Analyses of Hymenograndin and Hymenosignin[1]

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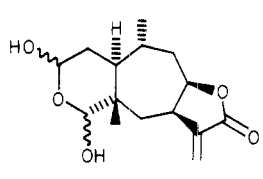
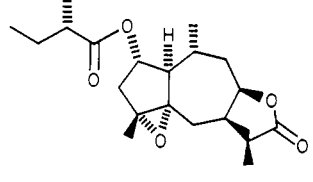
Hymenoxys insignis yielded the new guaianolide hymenosignin (**2**) and the new helenanolide acetylhymenograndin (**3b**). The structures were established by physical methods. These included X-ray analyses of **2** and hymenograndin (**3a**), whose previously postulated stereochemistry at C-2 and C-3 was found to require revision. Conformations of the various compounds and their bearings on the CD curves of substances related to **3a,b** are discussed, and a suggestion is made to account for the co-occurrence of hymenosignin and acetylhymenograndin.

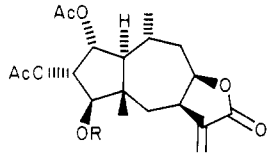
Several *Hymenoxys* species are important livestock poisons.[2] Their toxicity and mutagenic activity is due primarily to the secohelenanolide hymenovin (**1**) which is



1



2



3a, R = H
b, R = Ac

a mixture of hemiacetal epimers derived from a hypothetical hydrated seco dialdehyde.[3-5] Various related dilactones[6,7] and helenanolides[11-13] are other typical constituents of the genus. In the present paper we report isolation and structure determination of a new guaianolide, **2**, and a new helenanolide, **3b**, from the Mexican species *Hymenoxys insignis* (Gray ex Wats) Cockll. and comment on the possible significance of their co-occurrence. Hy-

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(7) Hymenovin, one of whose components has been named hymenoxon,[5] is easily converted[8] to the dilactones psilotropin (floribundin) and greenein which were isolated earlier[6] from several *Hymenoxys* species. The dilactones may therefore be artifacts. One of us has suggested[3,9] that the elusive "vermeeric acid" which is responsible for the toxicity of the South African *Geigeria* species may be the C-8 epimer of hymenovin and is converted upon extraction to the dilactone vermeerin.[6,10] The latter has been found in some toxic *Hymenoxys* species as well.[6]

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